(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 February 2003 (06.02.2003)

PCT

(10) International Publication Number
WO 03/010524 A1

(51) International Patent Classification[7]: G01N 21/64

(21) International Application Number: PCT/US02/23798

(22) International Filing Date: 25 July 2002 (25.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/307,682 25 July 2001 (25.07.2001) US

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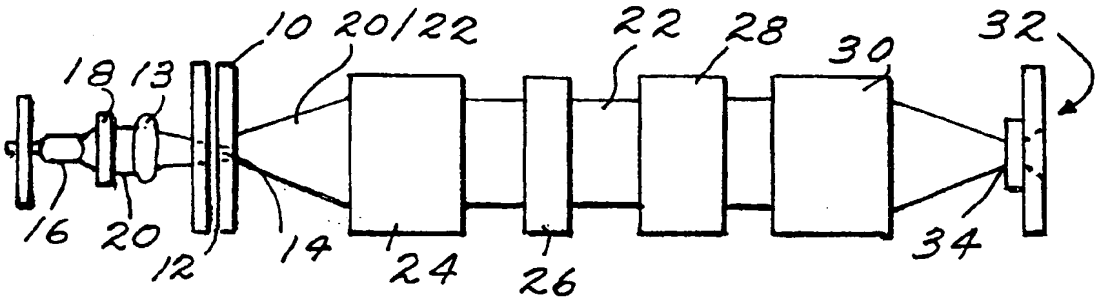
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TIME-DELAY INTEGRATION IN ELECTROPHORETIC DETECTION SYSTEMS



(57) Abstract: An apparatus for detecting analytes in a sample is provided. The apparatus includes: one or more channels (10) having a detection zone (14); one or more irradiation sources (16) disposed for irradiating the detection zone (14) with non-coherent radiation; a detector array (34) disposed for collecting light signals emitted from markers in the detection zone (14) excited by the radiation, the detector array (34) having an output; and a system coupled to the detector array (34) for effecting time delay integration of the charges on the detector array (34) corresponding to the light signals by accumulating the charges before reading the charges at the output of the detector array (34). Other apparatus and methods for detecting analytes in a sample are also provided.

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using an etched plate with capillary-sized grooves, or using a plurality of capillary tubes, where either type of device has multiple-channels used to increase throughput.

An electrophoretic apparatus and method that include a cost-effective and convenient source of irradiation and that do not compromise sensitivity or resolution would be desirable, especially in multiple-channel electrophoretic systems used to increase throughput.

SUMMARY OF THE INVENTION

According to various embodiments, the present invention can relate to an apparatus and method for detecting components, for example, analytes, in a sample.

According to various embodiments, an apparatus is provided for detecting analytes in a sample. According to various embodiments, the apparatus can include: one or more channels having a detection zone; one or more irradiation sources disposed for irradiating the detection zone with non-coherent radiation; a detector array disposed for collecting light signals emitted from markers in the detection zone excited by the radiation, where the detector array can have an output; and a system coupled to the detector array for effecting time delay integration of the charges on the detector array corresponding to the light signals by accumulating the charges before reading the charges at the output of the detector array.

According to various embodiments, an apparatus is provided for detecting analytes in a sample, wherein the apparatus can include: one or more channels having a detection zone; one or more irradiation sources disposed for irradiating the detection zone with radiation; a detector array disposed for collecting light signals emitted from markers in the detection zone excited by the radiation, where the detector array can have an output; and a system coupled to the detector array for effecting time delay integration of the charges on the detector array corresponding to the light signals by accumulating the charges before reading the charges at the output of the detector array, wherein the system for effecting time delay integration can do so by moving, relative to one another, the detector array and light signals from the detection zone.

According to various embodiments, an apparatus for detecting analytes in a sample is provided, wherein the apparatus can include: one or more channels having a detection zone; one or more irradiation sources disposed for irradiating the detection zone with radiation; a detector array disposed for collecting light signals emitted from markers in the detection zone excited by the radiation, where the detector array can have

excite markers responsive to the radiation and which emit light signals indicative of corresponding analytes; a detector array disposed for collecting the light signals produced by the markers and for producing charges corresponding to the light signals, where the detector array can have an output; modulating optics for modulating light between the at least one irradiation source and the detector array; and a time delay integration system for effecting, within the detector array, an accumulation of charges corresponding to light signals associated with at least one given analyte band before reading accumulated charges at the output of the detector array. The accumulation of charges can be effected during an integration time of the at least one given analyte band moving across the detection zone, by, for example, moving, relative to one another, the detector array and at least one of the detection zone and the modulating optics.

According to various embodiments, an apparatus is provided for detecting analytes in a sample, wherein the apparatus can include: a channel-defining member defining at least one channel therein having a detection zone; and a separating system coupled to the at least one channel for separating a sample containing analytes and disposed in contact with a migration medium in the at least one channel into analyte bands migrating along the at least one channel, wherein each analyte band can be detectable by the presence of a corresponding marker. The apparatus can further include at least one irradiation source disposed for irradiating the detection zone of the at least one channel with radiation to thereby excite markers responsive to the radiation for emitting light signals indicative of corresponding analytes. Together, the light signals can form an image corresponding to analyte bands migrating across the detection zone. The apparatus can also include a detector array disposed for collecting the light signals produced by the markers and for producing charges corresponding to the light signals, the detector array having an output; a re-imaging optical system disposed between the detection zone and the detector array for optically inverting an image produced by the light signals before the image is collected by the detector array; modulating optics for modulating light between the at least one irradiation source and the detector array; and a time delay integration system for effecting, within the detector array, an accumulation of charges corresponding to light signals associated with at least one given analyte band before reading accumulated charges at the output of the detector array, the accumulation being effected during an integration time of the at least one given analyte band moving across the detection zone.

several exemplary embodiments and together with the instant description, serve to explain the principles of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention may be more fully understood with reference to the accompanying drawing figures. The drawing figures are intended to illustrate exemplary embodiments of the present invention without limiting the scope of the present invention.

Fig. 1 is a schematic, side-elevational view of an electrophoresis arrangement according to various embodiments showing a channel-defining member in cross-section;

Fig. 2 is a schematic view of the image produced on the detector array of a detector at a time t using the arrangement of Fig. 1;

Fig. 3 is a view similar to Fig. 2 showing the image at a time $t+\Delta t$;

Fig. 4 is a schematic, front-elevational view of an electrophoresis arrangement according to various embodiments for the sequential use of multiple-color irradiation sources along with filters on a filter wheel;

Fig. 5a is a schematic, top-plan view of the arrangement of Fig. 4;

Fig. 5b is a schematic, side-elevational view of the arrangement of Fig. 4;

Figs. 6a through 6e are respective schematic views of images produced on the detector array of a detector, each image corresponding to light signals filtered through a respective filter on the filter wheel of Fig. 4;

Fig. 6f is a schematic representation of an electropherogram showing fluorescence intensity curves for each filter of the filter wheel in Fig. 4 during three signal readings by the detector;

Fig. 6g is a schematic representation of the intensity curves of Fig. 6f in aligned format for multicomponenting;

Fig. 6h is a schematic representation of multicomponented intensity curves for five different kinds of markers that can be used in the system of Fig. 4 based on the readings shown in Fig. 6f;

Fig. 6i is a graph of excitation efficiency versus wavelength for four exemplary markers;

Fig. 6j is a graph of fluorescence intensity versus wavelength for the exemplary markers of Fig. 6i;

Fig. 1 shows an exemplary embodiment of an electrophoresis device. As depicted in Fig. 1, the arrangement can include a channel-defining member 10 defining a channel 12 therein for the migration of an analyte sample. The channel-defining member 10 may include a cover plate with or without grooves, an etched plate defining one or more capillary sized grooves therein, or one or more capillary tubes. According to various embodiments, the channel-defining member can be an etched plate having a plurality of channels or grooves, or the channel-defining member can include a plurality of capillary tubes. The use of a plurality of channels can allow a large number of analyte samples to be measured simultaneously in order to increase throughput. As is well known, for electrophoresis to occur, opposing ends of channel-defining member 10, such as an electrophoretic plate or capillary tube, can be placed in contact with corresponding electrodes connected to a power supply for generating an electric field across the plate or tube. This field can cause the analyte to migrate from a loading site (not shown) for the plate or tube arrangement of the channel-defining member 10, toward a detection site or detection zone 14. The detection zone can encompass that zone on the channel that is irradiated by an irradiation source to excite markers, such as dye markers, used to label analytes in the sample.

An example of a marker compound is a dye marker. Any suitable marker, such as, for example, a fluorophore, can be used. Fluorophores useful according various embodiments can include those that can be coupled to organic molecules, particularly proteins and nucleic acids, and that can emit a detectable amount of radiation or light signal in response to excitation by an available excitation source. Suitable markers can encompass materials having fluorescent, phosphorescent, and/or other electromagnetic radiation emissions. Irradiation of the markers can cause them to emit light at varying frequencies depending on the type of marker used.

One class of markers provides signals for the detection of labeled extension and amplification products by fluorescence, chemiluminescence, and electrochemical luminescence (Kricka, L. in *Nonisotopic DNA Probe Techniques*, Academic Press, San Diego, pp. 3-28 (1992)). Chemiluminescent labels include 1,2-dioxetane compounds (U.S. Patent No. 4,931,223; and Bronstein, Anal. Biochemistry 219:169-81 (1994)). Fluorescent dyes useful for labeling probes, primers, and nucleotide 5'-triphosphates include fluoresceins, rhodamines (U.S. Patent Nos. 5,366,860; 5,936,087; and 6,051,719), cyanines (Kubista, WO 97/45539), and metal porphyrin complexes (Stanton,

that can overlap with the emission spectra of the markers being used. According to various embodiments, the conditioning filter may let through only light in the wavelength range of excitation light for one or more of the markers. Typically, any given LED emits excitation light in a spectral range. The range of wavelengths of the excitation light in turn may typically excite markers to emit light signals within a given spectral range in the detection zone. For the detection of light signals from the detection zone, one can block out that portion of the excitation light that would be in the same wavelength range as some or all of the light signals emitted from the detection zone. Otherwise, it can be difficult to determine which portion of the detected light is merely excitation light from the LED. The light passing through the conditioning filter 18 is conditioned light 20, as seen in Fig. 1. The excitation modulating optic can further include a focusing optical system 19. The conditioned light 20 can thereafter be focused by focusing optical system 19 for irradiating the analyte sample and corresponding markers in the detection zone 14. The thus irradiated marker or markers in turn emit light signals, such as through fluorescence, at frequencies specific to the irradiated marker, so as to present a peak intensity. For example, a dye excited by yellow light might have a fluorescence emission peak intensity at 610 nm corresponding to the orange portion of the spectrum, while a peak at about 460 nm is associated with the blue portion of the spectrum, and a peak at about 660 nm is associated with the red portion of the spectrum. It is noted that there are only a limited number of colors possible for efficient laser irradiation sources when compared with possible colors for efficient, non-coherent irradiation sources, such as LED's. The use of irradiation sources emitting non-coherent radiation in turn allows the use of a wider range of markers when compared with the use of lasers. By way of example, ROX, a known dye marker, is best excited at 590 nm. No laser, however, is particularly efficient at 590 nm. ROX is better excited by an LED emitting radiation at 590 nm.

The device of Fig. 1 further includes a collection modulating optics system that can include a collimating optical system 24, a wide bandpass filter 26, a transmission grating 28, and a re-imaging optical system 30. Emitted light 22 from the detection zone 14, and, in addition, conditioned light 20 passing through the detection zone 14, are collimated by a first optical component or system 24. In this respect, it is to be noted that the light from the detection zone includes the emitted light 22 and a portion of the conditioned light 20 passing through the detection zone. Alternatively, the excitation

Referring additionally now to Figs. 2 and 3, an image produced by moving analyte bands is recorded by the photo-detecting surface of CCD 32 (Fig. 1) at times t (Fig. 2) and t+Δt (Fig. 3). The photo-detecting surface 36 can be, according to an embodiment, part of the two dimensional detector array 34 shown above with respect to Fig. 1. Photo-detecting surface 36 can include a spectral axis as indicated by arrow λ on the figure, and a spatial axis along which the analyte bands move, as indicated by arrow M in Figs. 2 and 3. As further seen in Fig. 2, the image created by the light signals emitted by excited markers produces two bands 38 and 40 on photo-detecting surface 36 substantially in the red and blue regions of the spectrum, respectively, each band corresponding to a marker used to label, for example, a predetermined type of analyte. The bands are spectrally distributed along the spectral axis by transmission grating 28 in Fig. 1. At time t, as shown in Fig. 2, the charges produced on surface 36 present two respective peaks 46 and 48 on intensity profile 45. These peaks correspond to bands 38 and 40, respectively. As seen in Fig. 3, at time t+ Δt, both bands 38 and 40 have moved downward along the direction of migration M on the photo-detecting surface 36. Serial register 42 of the CCD 32 (Fig. 1) collects the charges accumulated for each analyte band during its integration time. All signals received from the detector can be converted from analog to digital and conveyed to a serial port for transmission to a multipurpose computer for storage and for further processing and analysis. The analog output can alternatively or additionally be sent directly to an output device for display or printing, or used for other purposes.

According to various embodiments, for example, as shown in the embodiment above, collection of the image is performed using time delay integration (TDI). In the CCD, the photogenerated charge in the photoactive elements or pixels can be transferred toward the serial register 42 one row at a time. The charge information in the serial row is then read by using a corresponding single on-chip amplifier or readout register 44 of the CCD. By way of example, for a 256 x 256 element CCD, each time a single imaging area is transferred to the serial register 42, 256 readouts of the thus transferred area are performed, each readout corresponding to a different spectral element. The above process continues until all 256 rows have been read 256 times.

Under a normal read-out approach, the motion of the images on the detector array 34 produces a blur. In TDI, according to various embodiments, the shutter is eliminated and the shifting of rows of the CCD is synchronized to the migration of the band of

effective integration time. In the context of various embodiments, the "effective integration time of an analyte band" therefore, as defined above, corresponds to the integration time for a portion of the wavelengths of the light signals in the analyte band, where the portion can include all of the wavelengths or a range thereof.

According to various embodiments, the use of TDI in collecting data points among other things addresses the problem of lowered irradiance when using irradiation sources emitting non-coherent light, such as LED's. The irradiance, that is, photons emitted per millimeters squared, is typically about a thousand times lower in LED's when compared with the irradiance of lasers. TDI, according to the present invention, among other things addresses the problem of lowered irradiance by allowing a longer period of time for the integration of signals from excited markers. Related to TDI is the use of a broad detection zone according to the present invention. In a non-TDI detection system, the detection zone is typically about one tenth of a millimeter squared. When using TDI according to various embodiments, the detection zone for one channel can be one hundred times larger, that is, about one millimeter squared, allowing a relatively larger number of markers to be excited and a larger number of data points to be integrated into a detector. The above principle of various embodiments can be equally applicable in instances where a plurality of channels are used, the detection zones of each of the respective channels being adapted to be irradiated by at least one irradiation source emitting non-coherent light.

For the purpose of accumulating charges to effect TDI, instead of shifting the charges on the CCD as a function of the migration of the analyte bands, various embodiments encompass within its scope moving the CCD itself and/or the image itself, that is, the light signals from the detection zone, as a function of migration of the analyte bands such that the result of such movement is the tracking of each analyte band by a continuously moving row of accumulating photogenerated charge on the CCD during the effective integration time of the analyte band. By way of example, to accomplish the desired result mentioned above, appropriate motors, gearing, belt drives, control units and power supplies can be used. For example, a linear actuator can be used to translate the re-imaging optical system 30 and/or the CCD itself to minimize blurring. The image can in this way be made to be stationary on the CCD throughout the integration time. In such a case, a frame transfer CCD can be used. A frame transfer CCD has a parallel register that can be composed of two CCD's arranged in tandem. The CCD register

excitation light for one or more of the markers. The conditioning filters 18 each substantially block predetermined ranges of wavelengths of light emitted by the corresponding LED. The predetermined ranges correspond to wavelengths of light that can overlap with the emission spectra of the markers being excited by the corresponding LED. The function of each optical system 19 for focusing is to focus the conditioned light from the conditioning filter onto the detection zone 14, which, in the embodiment of Figs. 4, 5a and 5b, corresponds to a respective detection zone for each of the shown capillary tubes. Excited markers in detection zone 14 thereafter emit light signals in the form of emitted light 22. The light from the detection zone, as in the case of the first-mentioned embodiment of the present invention described in relation to Fig. 1, includes the emitted light 22, and, in addition, a portion of the conditioned light 20 passing through the detection zone.

According to various embodiments, the light from the detection zone, labeled 20 and 22 in Fig. 5a, can be collimated by collimating optical system 24. The light thus collimated is thereafter passed through a corresponding bandpass filter 50' on filter wheel 60 as shown in broken lines in Figs. 5a and 5b. It is noted that the filters in Figs. 5a and 5b have been shown in phantom (broken lines) because, in those figures, the depiction of the filter wheel 60 is not cross-sectional, but rather represents plan views thereof.

Referring to Fig. 4, the filter wheel is shown in more detail as supporting therein a plurality of bandpass filters 51, 53, 55, and 57. Each of the bandpass filters is adapted to let through, substantially exclusively, predetermined wavelengths of light from the detection zone corresponding to a portion of the wavelengths of the light signals emitted by an associated marker. According to various embodiments, there is a bandpass filter provided for each associated marker. The portion of the wavelengths of the light signals can include all of the light signals, or it can include, for each marker, a range of wavelengths about the peak intensity of light signals. For example, the range of wavelengths about a peak intensity of emitted light signals can be between about 5% to about 20% of wavelengths on each side of the peak for a given marker, or it can include the range of wavelengths at about half of the intensity of the peak, generally called "full width at half max." Thus, by way of example, bandpass filter 51 is adapted to filter therethrough light signals emitted by given markers responsive to LED 50. In Figs. 5a and 5b, the apparatus according to various embodiments is depicted in a mode where LED 50 irradiates the detection zone 14. However, it is clear that any of the shown

one of the irradiation sources 50, 52, 54, and 56 appropriately mounted to allow fragment analysis.

Referring now to Figs. 6a through 6e, these figures depict images produced by moving analyte bands on the detector array 34 of CCD 32 shown in the embodiment of Figs. 4, 5a and 5b. Images are shown for each of the markers used to label the analytes and which are responsive to excitation by a given one of the irradiation sources 50, 52, 54, and 56. Each shown frame of photo-detecting surface 36 in Figs. 6a through 6e shows two lanes of analyte bands each corresponding to one of the two capillaries of channel-defining member 58. The bands move along the direction of migration M, and are shown as being limited on each side thereof in the spectral direction by virtue of the light from the markers having been filtered through a corresponding bandpass filter. At the right of each frame is shown an intensity profile 45 corresponding to the capillary for which the charges are produced on photo-detecting surface 36 on the right lane thereof.

According to various embodiments, the intensity profiles are, according to known methods, aligned and combined, and thereafter can be multicomponented in order to account for any spectral overlap. Similar to the embodiment of Fig. 1, the serial register of the CCD 32 in the embodiment of Figs. 4, 5a, and 5b collects the charges accumulated for each analyte band during its integration time. All signals received from the detector can be converted from analog to digital and conveyed to a serial port for transmission to a multipurpose computer for storage and for further processing and analysis. Alternatively or additionally, the analog output could be sent directly to an output device for display or printing. By way of example, a multipurpose computer may be used to perform the multicomponenting process. Multicomponenting is a process that is known to a person skilled in the art, and that can involve a spectral calibration within a multicomponenting software program. The spectral calibration can be obtained through a predetermined signature matrix corresponding to each marker. Each signature matrix provides a signature snapshot of the intensity of light signals by a given marker as a function of the wavelengths of those light signals. By virtue of the signature matrices, a combination of intensity curves for a given wavelength band emitted from the detection zone can be broken down into its components corresponding to light signals emitted by individual ones of the markers. In this way, a relatively accurate assessment of the light signals by respective ones of the markers can be made for the detection process.

signals that go beyond the CCD's full well capacity. In this way, analyte concentrations may be increased while still allowing the CCD to reliably detect signal levels without saturation. According to various embodiments, there is a trade-off between the use of fewer cycles amounting to longer integration times (such as, for example, 5 seconds) and the use of more cycles amounting to shorter integration times (such as, for example, 1 second). Longer integration times are useful where the noise level is relatively high and where the sensitivity of the system needs to be increased in light of the same. On the other hand, where the noise level in the system is relatively low, multiple reads may be taken of signals from the same marker, and the read signals may thereafter be multicomponented, the CCD in this way allowing the detection of brighter peaks without going off-scale.

By way of example, a frame transfer CCD may be controlled to collect the light signals corresponding to the blue marker during integration time t while the LED exciting primarily the blue marker irradiates the detection zone. Thereafter, the entire CCD is read out. The filter wheel is then switched to the bandpass filter associated with the green marker, and the LED exciting primarily the green marker irradiates the detection zone. The CCD then collects the light signals corresponding to the green marker during integration time t. The entire CCD is then read out. The filter wheel is then switched to the bandpass filter associated with the yellow marker, and the LED exciting primarily the yellow marker irradiates the detection zone. The CCD then collects the light signals corresponding to the yellow marker during integration time t, and the entire CCD is thereafter read out. The above process can then be repeated for all five markers in this example and as described in relation to Figs. 4, 5a and 5b. As suggested above, in this example, the band takes about five times the integration time from the top of the frame transfer CCD to the bottom thereof, that is, to the readout register. Each readout of the CCD corresponds to one marker. All of the readouts can then be aligned and combined in a known manner for multicomponenting.

An example of the manner in which multicomponenting may be effected is shown in Figs. 6f through 6h. Here, it is assumed that filters 51, 53, 55, 57, and FD let through wavelengths of light in the blue, green, yellow, red and "fifth" portions of the spectrum. The "fifth" portion may, for example, be in the orange range of the spectrum. Fig. 6f is a schematic representation of an electropherogram showing fluorescence intensity curves for each filter of the filter wheel in Fig. 4 during three readings of the

four different dye markers that can be used in oligosynthesis, namely, 5-FAM, JOE, TAMRA, and ROX. These dye markers are exemplary of those that can be used in various embodiments where a plurality of dye markers are to be used, such as the embodiments shown in Figs. 4, 5a, 5b, and 7.

As seen in Fig. 6i, the x-axis corresponds to wavelengths, expressed in nanometers or nm, emitted by an irradiation source, and the y-axis corresponds to the percentage of excitation efficiency. Here, it can be easily appreciated that 5-FAM has its maximum absorbance, corresponding to its peak percent excitation efficiency, at about 490 nm. The maximum absorbance at a given wavelength indicates that the dye marker being considered fluoresces at its peak fluorescence intensity when it is irradiated at the given wavelength. As further seen in Fig. 6i, JOE has a maximum absorbance at about 526 nm, TAMRA has a maximum absorbance at about 560 nm, and ROX has a maximum absorbance at 588 nm. The wavelengths on the x-axis could be emitted by any irradiation source, such as, for example, an LED. Fig. 6i also shows that, where a dye marker, such as 5-FAM, is being irradiated at its maximum absorbance wavelength, other dye markers, such as, for example, JOE, TAMRA, and ROX, do exhibit some absorbance, although to a lesser extent when compared to 5-FAM.

Referring now to Fig. 6j, the x-axis corresponds to the wavelengths of fluorescent light, expressed in nm, emitted by excited dye markers. The y-axis corresponds to the percentage of fluorescence intensity. As seen in Fig. 6j, 5-FAM has a peak fluorescence intensity at about 522 nm, JOE has a peak fluorescence intensity at about 554 nanometers, TAMRA has a peak fluorescence intensity at about 582 nm, and ROX has a peak fluorescence at about 608 nm. The wavelengths on the x-axis are emitted by the four mentioned dye markers. Fig. 6j also shows that, where a dye marker, such as TAMRA, fluoresces at its peak fluorescence intensity, other dye makers, such as 5-FAM, JOE, and ROX, also fluoresce, although at lesser fluorescence intensities.

When light is emitted from the dye markers in the detection zone at various colors, each dye marker can be excited efficiently, and further in a way that will allow its detection by way of its unique spectral signature. When two dye markers exhibit fluorescence intensity peaks that are close together, that is, for example, when the difference between the fluorescence intensity peaks of two dye markers is less than about 30 nm, there is a high level of overlap of the light emitted by those two dye markers. A high level of overlap makes it harder to distinguish between the light emitted by the two

of Fig. 7 differs from the embodiment of Figs. 4, 5a, and 5b in a number of respects. The premise behind the embodiment of Fig. 7 is to allow a reading of light signals having wavelengths in differing frequency ranges on the same array of the detector, the charges corresponding to the generated light signals being spatially offset as a function of the bandpass filter being used in connection with the markers emitting those light signals. Advantageously, such an embodiment allows a continuous reading of the accumulated charges on the detector array during time delay integration or TDI, rather than a frame-by-frame reading as in the case of the embodiment of the present invention described above with reference to Figs. 4, 5a, and 5b.

In the embodiment of Fig. 7, the irradiation sources, together with associated optics such as the conditioning filter 18 and the optical system for focusing 19, can be provided on an irradiation source wheel 61 as shown. As seen in Fig. 7, components of the apparatus that are similar to those in the embodiment of Fig. 1 have been labeled with the same reference numerals, such as conditioning filter 18, focusing optical system 19, collimating optical component or system 24, and re-imaging optical component or system 30. The irradiation wheel 61 is rotatable to selectively position each respective one of the irradiation sources and associated optics to irradiate detection zone 14. Four irradiation sources similar to sources 50, 52, 54, and 56 in Figs. 4, 5a, and 5b, can be provided. In addition, a filter wheel 61' can be provided, similar to filter wheel 60 in Figs. 4, 5a, and 5b. The rotation of both wheels 61 and 61' can be effected by the provision of a filter wheel drive 62 similar to the filter wheel drive of the embodiment of Figs. 4, 5a, and 5b described above. According to various embodiments, the two wheels 61 and 61' can further be coupled to one another and to the filter wheel drive 62 by way of a rotatable shaft 63 as shown. It is noted that various embodiments encompass within its scope the provision of a plurality of irradiation sources that are not necessarily provided on an irradiation wheel, or the provision of an arrangement where the two wheels 61 and 61' are not coupled to one another by a shaft, but are rather actuated independently by their own respective wheel drives.

In the embodiment of Fig. 7, the apparatus is provided with an offset system 64, which may be disposed either on the filter wheel 61' in association with a corresponding bandpass filter, or coupled to at least one of the detector 32, the modulating optics, and the detection zone 14, for spatially offsetting the light signals impinging upon the array of the detector by a predetermined amount as a function of the bandpass filter being

light signals should be situated with respect to the detector array itself, and with respect to light signals in different wavelength frequency ranges. Thus, where individual mechanisms 66 are used in conjunction with a corresponding bandpass filter to offset the light emerging therefrom, each mechanism 66 is chosen according to the frequency ranges of wavelengths that the bandpass filter lets through. In the alternative, where offset control device 67 is used, the offset control may be programmed to offset at least one of the detection zone 14, the modulating optics, and the detector array 34, with respect to one another by the predetermined amount. The offsetting could, by way of example, be accomplished by moving either the detection zone 14, the modulating optics, or the detector array 34, in a translational motion by the predetermined amount, the image created by the light signals being spatially offset correspondingly. The embodiment shown in Fig. 7 can involve the use of a plurality of LED's similar to those used in the embodiment of Figs. 4, 5a, and 5b. The offset amount should be sufficient be prevent overlap of the images from each bandpass filter.

It is noted that offset system 64, including offset mechanisms 66, or, in the alternative, offset control device 67, are shown in broken lines in Fig. 7 in order to suggest that mechanisms 66 and offset control device may be used as alternatives of the offset system 64. It is further noted that various embodiments encompass within its scope instances where both alternatives, that is, mechanisms 66 and offset control device 67, are used in conjunction with one another. Moreover, the modulating optics, according to various embodiments, comprise at least one of conditioning filter 18, focusing optical system 19, collimating optical system 24, and re-imaging optical system 30, encompasses any devices or system for achieving the functions associated with the components listed above as would be within the knowledge of persons skilled in the art. In addition, with respect to offset control device 67, where the instant disclosure describes a coupling of device 67 to the modulating optics, what is meant is that the offset control device 67 is coupled to at least one of the components of the modulating optics. It is further to be noted that, although the embodiments of Figs. 1 and 4-5b depict a set of two capillaries being analyzed, various embodiments encompass a detection zone defined by any suitable channel-defining member, such as any number of capillaries, any number of channels in an etched plate, and even a slab plate. According to various embodiments, the channel-defining member can assume any orientation according to application needs, such as a horizontal orientation or a vertical orientation. Moreover,

application basis. To the extent that the embodiment of Fig. 7 includes an offset system for spatially offsetting light signals in differing frequency ranges, the embodiment allows a continuous reading of accumulated charges by the detector, thereby making possible a continuous time delay integration of the light signals from the analytes in a sample into the detector array 34. Unlike the embodiment of Figs. 4, 5a, and 5b, where the accumulated charges corresponding to each range of wavelengths of light signals are read and discarded before charges for the next range of wavelengths are read, the embodiment of Fig. 7 allows the accumulated charges from each wavelength range to be read in a continuous fashion.

Referring now to Fig. 8, the image produced by the moving analyte bands on the array of detector (CCD) 32 is shown for each of the wavelength ranges and each of the two capillaries 58. In the shown image, each range of wavelengths is assigned an arbitrary color, the ones shown therefore being arbitrarily referred to as "blue," "green," "yellow," "red," and "fifth." For each color, the column on the left corresponds to charges produced by light signals from one capillary, and the column on the right to charges produced by the next capillary. As shown, the array features charges generated by light signals across the color axis λ, offset with respect to one another by offset system 64 in the manner previously described. Although in the shown embodiment, five arbitrary colors are given by way of example, the number of wavelength ranges used will be dependent on the particular application, and can range from one to as many as the system supports. It is noted that the λ axis is referred to here as the "color axis" rather than the "spectral axis," because there is no need for the colors, one for each bandpass filter, to be arranged from shorter to longer wavelengths. According to the embodiment as shown in Fig. 7, to the extent that charges from light signals of differing wavelengths may be produced on the same array of light signals so as to be spatially offset with respect to one another, as seen in Fig. 8, those charges may be accumulated during the integration time and read by the detector on a continuous basis. This eliminates the need to take the time to shut off the detector in order to allow a reading of charges in a given range of wavelengths on a frame-by-frame basis, and/or eliminates the need for a frame transfer CCD. In addition, the above embodiment facilitates a longer integration time, simpler data output and a less complex CCD.

According to various embodiments, the detection zone, including one or more channels, or a slab gel, may be irradiated with multiple-color irradiation sources, such as

passing therethrough, are, among other things, functions of the LED set being used, and, to the extent that any number of LED's may be used emitting light in any ranges of frequencies, the conditioning filter is chosen accordingly.

It is further to be noted that the conditioning filter of various embodiments may include a single conditioning filter, or a series of conditioning filters capable of filtering the light from the LED's as previously described. The conditioned light is, as previously described above in relation to Figs. 1 and 7, focused onto the detection zone of an electrophoretic detection system. The light emitted by markers in the detection zone is then passed through a bandpass filter. The function of the bandpass filter is to let through, substantially exclusively, predetermined wavelengths of light from the detection zone corresponding to a portion of the wavelengths of the light signals emitted by an associated set of markers, to thereby produce filtered light. The bandpass filter's function is to let through only a portion of the light emitted by an associated set of markers, and not the excitation light by the LED's that passes the detection zone. The portion of the wavelengths of the light signals can include all of the light signals, or it can include, for each marker, a range of wavelengths about the peak intensity of light signals. For example, the range of wavelengths about the peak intensity of light signals can be between about 5% to about 20% of wavelengths on each side of the peak wavelength of a given marker, or it can include a range of wavelengths at about half of the intensity of the peak wavelength, or "full width at half max." The collection of the predetermined wavelengths can be performed using a dispersion approach, such as the approach shown in Fig. 1, or by additional bandpass filters as shown in Fig. 4.

As seen in Fig. 9c, where percent light transmission is plotted versus wavelength, the particular bandpass filter being used, the behavior of which is shown in the figure, lets through light corresponding to the "blue," "green," "yellow," "red," and "orange" markers. The regions or zones corresponding to the excitation light by the LED's are blocked. As noted previously with respect to the conditioning filter, the bandpass filter in various embodiments may include a single bandpass filter, or a series of bandpass filters capable of filtering the light from the LED's as previously described.

Fig. 9d plots relative emission intensity versus wavelength, expressed in nm, for the light let through by the bandpass filter. Fig. 9d, in effect, provides a breakdown, by wavelength, of the light transmitted through the bandpass filter. As shown in Fig. 9d, the markers being excited by the LED's used in the example of Figs. 9a through 9d emit light

depicted by reference numeral 78. Data can be collected from frame 74a from the top of the frame to the bottom of the frame. Some of the light is blocked by the mask, causing a variable collection efficiency as suggested by Fig. 12b. The integration of the accumulated charges occurs in the embodiment of the present invention shown in Figs. 10-12b on a frame-by-frame basis as previously explained in relation to Figs 4, 5a, and 5b, the frame data being combined in a known manner to create an electropherogram. It is further possible, according to various embodiments, to abut the frames, that is, to eliminate any distance between them so as to combine the resulting images on detector array 34. According to various embodiments, the above arrangement can allow the separation and detection of light signals from multiple-channels while permitting the simultaneous irradiation of those channels with multiple-color irradiation sources. The above is made possible through the use of a single camera instead of one camera per channel, the single camera maintaining a spatial separation of light signals from each masked channel.

According to various embodiments, the modulating optics that can be used in the electrophoresis arrangements are disclosed in U.S. Application Serial Number 09/564,790, the content of which is incorporated herein in its entirety by reference. In particular, in the above-referenced application, the modulating optics shown in Fig. 1, using the cat's eye aperture of Fig. 24, can be useful in electrophoresis arrangements according to the various embodiments.

Various embodiments can further pertain to an apparatus for detecting analytes in a sample, and can comprise: means defining at least one channel therein having a detection zone; means for separating a sample containing analytes and disposed in contact with a migration medium disposed within the at least one channel into analyte bands migrating along the at least one channel, wherein each analyte band is detectable by the presence of a marker; means for irradiating the detection zone with non-coherent radiation, that can thereby excite markers responsive to the radiation and which emit light signals indicative of corresponding analytes; means for detecting the light signals by collecting the light signals that can thereby produce charges corresponding to the light signals; means for effecting a time delay integration of the light signals within the detector array by accumulating within the detector array the charges corresponding to light signals associated with at least one given analyte band during an integration time of the at least one given analyte band moving across the detection zone; and means for

WHAT IS CLAIMED IS:

1. An apparatus for detecting analytes in a sample, comprising:

one or more channels having a detection zone;

one or more irradiation sources disposed for irradiating the detection zone with non-coherent radiation;

a detector array disposed for collecting light signals emitted from markers in the detection zone excited by the radiation, the detector array having an output; and

a system coupled to the detector array for effecting time delay integration of charges on the detector array corresponding to the light signals by accumulating the charges before reading the charges at the output of the detector array.

2. The apparatus according to claim 1, wherein the system for effecting time delay integration accumulates the charges by at least one of (a) shifting the charges on the detector array, and (b) moving, relative to one another, the detector array and light signals from the detection zone.

3. The apparatus according to claim 1, wherein the one or more irradiation sources comprises one or more light emitting diodes.

4. The apparatus according to claim 1, further comprising modulating optics disposed between the detection zone and the detector array, the modulating optics comprising a relay lens system having a collimating lens and a re-imaging lens.

5. The apparatus according to claim 4, wherein the modulating optics further include a conditioning filter disposed between the one or more irradiation sources and the detection zone.

6. The apparatus according to claim 5, wherein the modulating optics further include a focusing lens disposed between the conditioning filter and the detection zone.

7. The apparatus according to claim 4, wherein the modulating optics further include a transmission grating disposed between the focusing lens and the re-imaging lens.

8. The apparatus according to claim 4, wherein the modulating optics further include a filter disposed between the detection zone and the detector array for filtering through only the light signals.

9. An apparatus for detecting analytes in a sample, comprising:

one or more channels having a detection zone;

responsive to the radiation and which emit light signals indicative of corresponding analytes;

a detector array disposed for collecting the light signals produced by the markers and for producing charges corresponding to the light signals, the detector array having an output;

modulating optics for modulating light between the at least one irradiation source and the detector array; and

a time delay integration system for effecting, within the detector array, an accumulation of charges corresponding to light signals associated with at least one given analyte band before reading accumulated charges at the output of the detector array, the accumulation being effected during an integration time of the at least one given analyte band moving at least partially across the detection zone.

13. The apparatus according to claim 12, wherein the time delay integration system accumulates the charges by at least one of (a) shifting the charges on the detector array, and (b) moving, relative to one another, the detector array and light signals from the detection zone.

14. he apparatus according to claim 12, wherein the at least one irradiation source comprises at least one light emitting diode.

15. The apparatus according to claim 12, further comprising a sample containing at least one analyte therein labeled with a marker and being in contact with the migration medium.

16. The apparatus of claim 12, wherein said marker is a dye marker.

17. The apparatus according to claim 12, wherein the modulating optics comprises a respective conditioning filter for each irradiation source of the at least one irradiation source, each respective conditioning filter being effective for substantially blocking predetermined wavelengths of light emitted by a respective one of the at least one irradiation source to thereby produce conditioned light, the predetermined wavelengths being those wavelengths not overlapping with the emission spectra of the markers responsive to the respective one of the at least one irradiation source.

18. The apparatus according to claim 17, wherein the modulating optics comprises an optical system for focusing the conditioned light onto the detection zone.

at least one of a respective long pass filter and a respective bandpass filter for each of the at least one irradiation source for letting through, substantially exclusively, predetermined wavelengths of the light signals from the detection zone corresponding to a portion of the light signals emitted by an associated set of markers, to thereby produce filtered light.

25. The apparatus according to claim 24, wherein the modulating optics further comprises an optical system for collimating light from the detection zone to thereby generate collimated light.

26. The apparatus according to claim 24, wherein the modulating optics further comprises a re-imaging optical system for focusing the filtered light onto the detector array.

27. The apparatus according to claim 20, wherein:

the at least one irradiation source comprises a plurality of light emitting diodes each emitting light in a respective predetermined frequency range; and

the respective bandpass filter comprises a plurality of bandpass filters each being associated with a respective one of the plurality of light emitting diodes, each respective bandpass filter being effective for letting through, substantially exclusively, predetermined wavelengths of light from the detection zone corresponding to a portion of the wavelengths of the light signals emitted by an associated set of markers to thereby produce filtered light.

28. The apparatus according to claim 27, further comprising an offset system for spatially offsetting the filtered light from each respective bandpass filter by a predetermined amount as a function of said each respective bandpass filter such that an image on the detector array is produced by charges that are spatially offset from one another.

29. The apparatus according to claim 28, wherein the offset system comprises a plurality of offset mechanisms each associated with a respective one of the bandpass filters.

30. The apparatus according to claim 29, wherein each offset mechanism includes one of a glass plate, a grating, and a mirror.

31. The apparatus according to claim 28, wherein the offset system is adapted to effect a translational movement of at least one of the detector array, the modulating

a mechanism for duty cycling the filter wheel and the irradiation wheel to produce charges on the detector array corresponding to an irradiation of the detection zone by any combination of the light emitting diodes during each integration time interval.

40. The apparatus according to claim 12, wherein the detector array comprises a two-dimensional charge-coupled device.

41. The apparatus according to claim 12, wherein:

the at least one channel comprises a plurality of channels;

the at least one irradiation source includes a plurality of light emitting diodes adapted to simultaneously irradiate the plurality of channels; and

the apparatus further comprises masks to selectively mask the channels such that the light signals from respective detection zones thereof are distinct.

42. An apparatus for detecting analytes in a sample, comprising:

a channel-defining-member defining at least one channel therein having a detection zone;

a separating system coupled to the at least one channel for separating a sample containing analytes into analyte bands migrating along the at least one channel, wherein each analyte band is detectable by the presence of a corresponding marker;

at least one irradiation source disposed for irradiating the detection zone of the at least one channel with radiation to thereby excite markers responsive to the radiation and which emit light signals indicative of corresponding analytes;

a detector array disposed for collecting the light signals produced by the markers and for producing charges corresponding to the light signals, the detector array having an output;

modulating optics for modulating light between the at least one irradiation source and the detector array; and

a time delay integration system for effecting, within the detector array, an accumulation of charges corresponding to light signals associated with at least one given analyte band before reading accumulated charges at the output of the detector array, the accumulation being effected during an integration time of the at least one given analyte band moving across the detection zone by moving, relative to one another, the detector array and at least one of the detection zone and the modulating optics.

the at least one irradiation source comprises a plurality of light emitting diodes each emitting light in a predetermined frequency range; and

the modulating optics comprises a plurality of bandpass filters each associated with a respective one of the light emitting diodes, each respective bandpass filter being effective for letting through, substantially exclusively, predetermined wavelengths of light from the detection zone corresponding to a portion of the wavelengths of the light signals emitted by an associated set of markers to thereby produce filtered light.

50. The apparatus according to claim 49, further comprising an offset system for spatially offsetting the filtered light from each respective bandpass filter by a predetermined amount as a function of said each respective bandpass filters such that an image on the detector array is produced by charges that are spatially offset from one another.

51. An apparatus for detecting analytes in a sample, comprising:

a channel-defining-member defining at least one channel therein having a detection zone;

a separating system coupled to the at least one channel for separating a sample containing analytes into analyte bands migrating along the at least one channel, wherein each analyte band is detectable by the presence of a corresponding marker;

at least one irradiation source disposed for irradiating the detection zone of the at least one channel with radiation to thereby excite markers responsive to the radiation for emitting light signals indicative of corresponding analytes, the light signals together forming an image corresponding to analyte bands migrating across the detection zone;

a detector array disposed for collecting the light signals produced by the markers and for producing charges corresponding to the light signals, the detector array having an output;

a re-imaging optical system disposed between the detection zone and the detector array for optically inverting an image produced by the light signals before the image is collected by the detector array;

modulating optics for modulating light between the at least one irradiation source and the detector array; and

a time delay integration system for effecting, within the detector array, an accumulation of charges corresponding to light signals associated with at least one given analyte band before reading accumulated charges at the output of the detector array, the

predetermined wavelengths of light from the detection zone corresponding to a portion of the wavelengths of the light signals emitted by an associated set of markers to thereby produce filtered light.

57. The apparatus according to claim 56, further comprising an offset system for spatially offsetting the filtered light from each respective bandpass filter by a predetermined amount as a function of said each respective bandpass filter such that an image on the detector array is produced by charges that are spatially offset from one another.

58. A method for detecting analytes in a sample, comprising the steps of:

providing a channel-defining-member defining at least one channel therein having a detection zone;

providing a migration medium within the at least one channel;

separating a sample containing analytes and disposed in contact with the migration medium into analyte bands migrating along the at least one channel, wherein each analyte band is detectable by the presence of a marker;

irradiating the detection zone with non-coherent radiation using at least one irradiation source thereby exciting markers responsive to the radiation for emitting light signals indicative of corresponding analytes;

detecting the light signals produced by the markers by collecting the light signals on a detector array to produce charges on the detector array corresponding to the light signals;

modulating light between the at least one irradiation source and the detector array;

effecting a time delay integration of the light signals within the detector array by accumulating the charges within the detector array corresponding to light signals associated with at least one given analyte band during an integration time of the at least one given analyte band moving across the detection zone; and

reading the accumulated charges.

59. The method according to claim 58, wherein the step of accumulating the charges within the detector array includes at least one of the steps of shifting the charges on the detector array and moving, relative to one another, the detector array and light signals from the detection zone.

providing a channel-defining-member defining at least one channel therein having a detection zone;

providing migration medium within the at least one channel;

separating a sample containing analytes and disposed in contact with the migration medium into analyte bands migrating along the at least one channel, wherein each analyte band is detectable by the presence of a marker;

irradiating the detection zone using at least one irradiation source generating radiation of such wavelength as to thereby excite markers responsive to the radiation for emitting light signals indicative of corresponding analytes;

detecting the light signals produced by the markers by collecting the light signals on a detector array to produce charges on the detector array corresponding to the light signals;

modulating light between the at least one irradiation source and the detector array using modulating optics;

effecting a time delay integration of the light signals within the detector array by accumulating the charges within the detector array corresponding to light signals associated with at least one given analyte band during an integration time of the at least one given analyte band moving across the detection zone, wherein the accumulation is effected by moving, relative to one another, the detector array and at least one of the detection zone and the modulating optics; and

reading the accumulated charges.

66. The method according to claim 65, further comprising the step of providing a sample containing at least one analyte therein labeled with a marker and being in contact with the migration medium.

67. The method according to claim 65, wherein the step of modulating comprises:

substantially blocking predetermined wavelengths of light emitted by a respective irradiation source of the at least one irradiation source to thereby produce conditioned light, the predetermined wavelengths being those wavelengths not overlapping the emission spectra of the markers responsive to the respective one of the at least one irradiation source;

focusing the conditioned light onto the detection zone;

collimating light from the detection zone to thereby generate collimated light; and

light signals associated with at least one given analyte band during an integration time of the at least one given analyte band moving across the detection zone; and

means for reading the accumulated charges.

72. An apparatus for detecting analytes, comprising:

a plurality of elongate channels;

a non-coherent light source;

an excitation-light pathway extending from said light source to said channels;

focusing optics disposed along said excitation-light pathway; and

a charge-coupled device optically coupled to said channels,

wherein said charge-coupled device is configured to operate in time-delay integration mode.

73. The apparatus of claim 72, wherein said channels are defined by capillary tubes or grooved plates.

74. The apparatus of claim 72, further comprising a separation medium supported by said channels.

75. The apparatus of claim 72, including at least four co-extensive channels.

76. The apparatus of claim 72, further comprising modulating optics disposed along an optical path extending between said charge-coupled device and said channels.

77. The apparatus of claim 72, wherein said light source is a light-emitting diode.

78. An apparatus for detecting analytes, comprising:

a plurality of elongate channels, each having an inlet end and an outlet end;

a detection zone located at one or both of (i) along said channels and (ii) outside of said channels on a side of said outlet ends opposite the inlet ends;

a non-coherent light source and associated focusing optics configured for irradiating at least a portion of said detection zone with non-coherent light; and

a charge-coupled device optically coupled to said detection zone;

wherein said charge-coupled device is configured to operate in time-delay integration mode.

79. The apparatus of claim 78, wherein said detection zone is located along said channels.

80. The apparatus of claim 78, wherein said detection zone is located outside of said channels on a side of said outlet ends opposite the inlet ends.

Fig. 1.

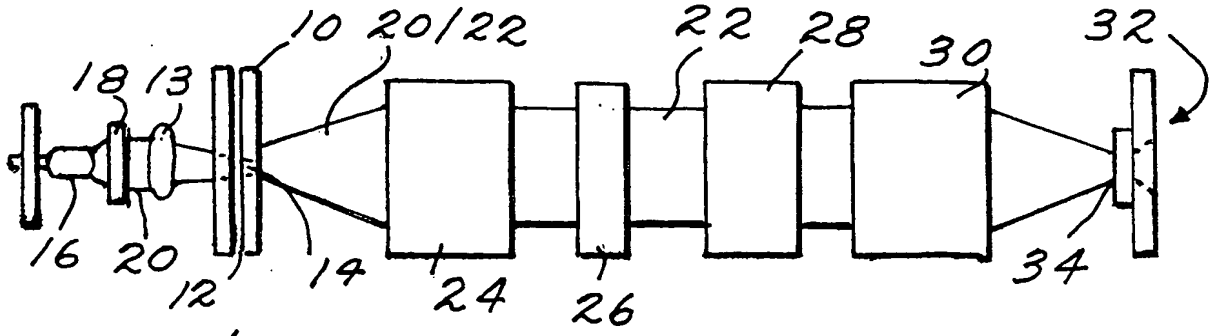


Fig. 2.

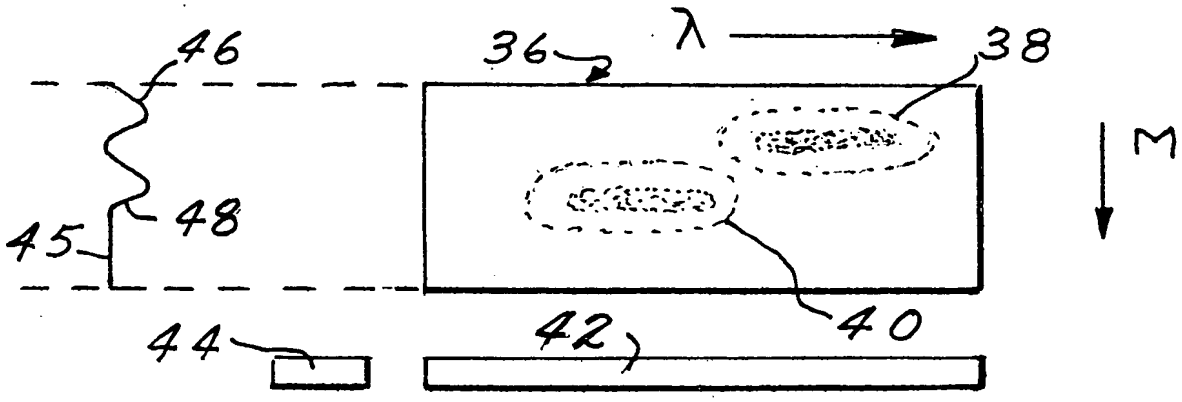
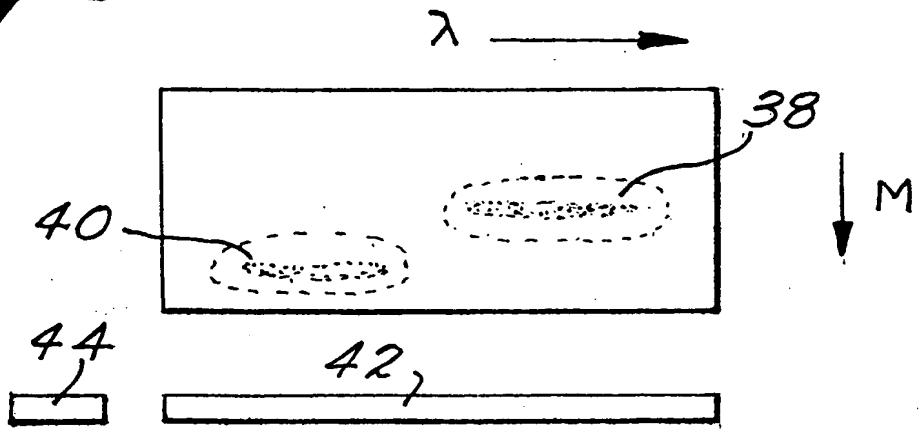


Fig. 3.



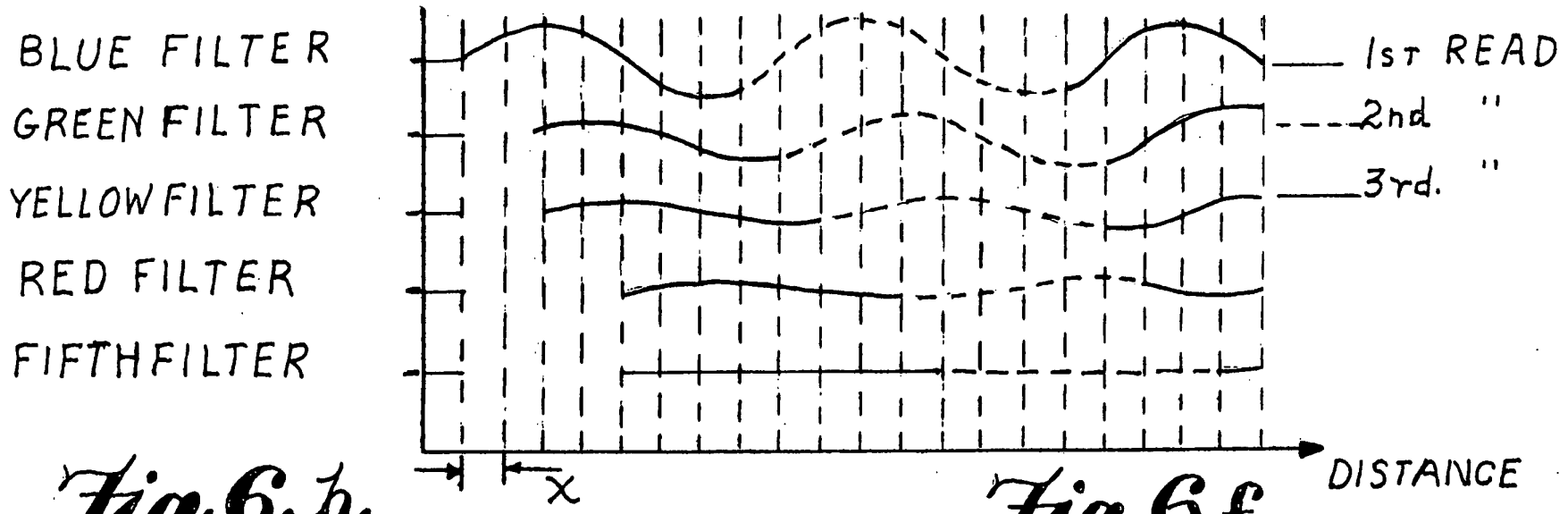


Fig. 6. h.

Fig. 6 f.

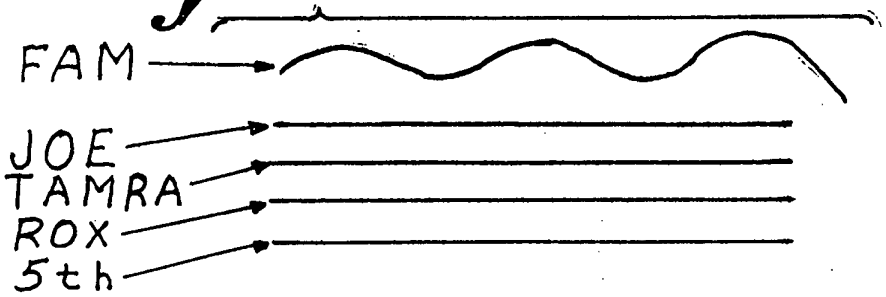
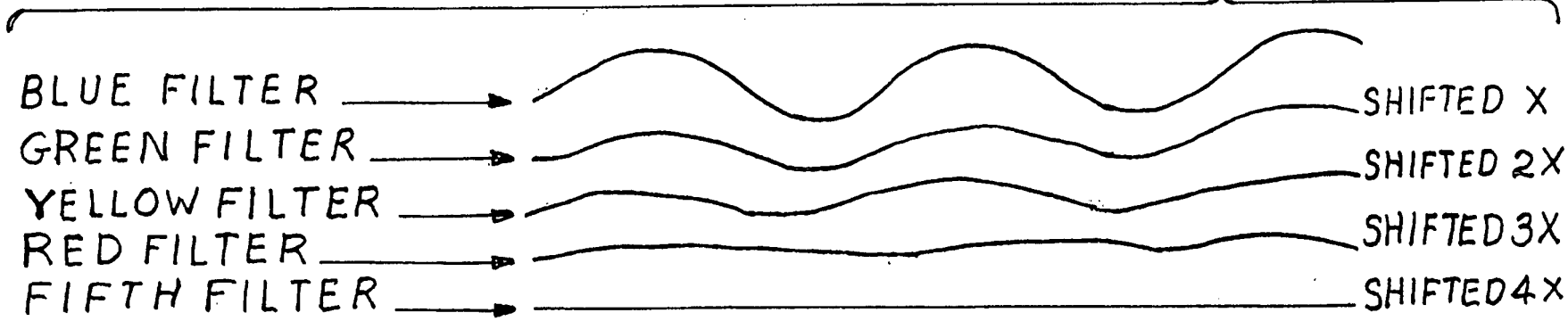


Fig. 6 g.



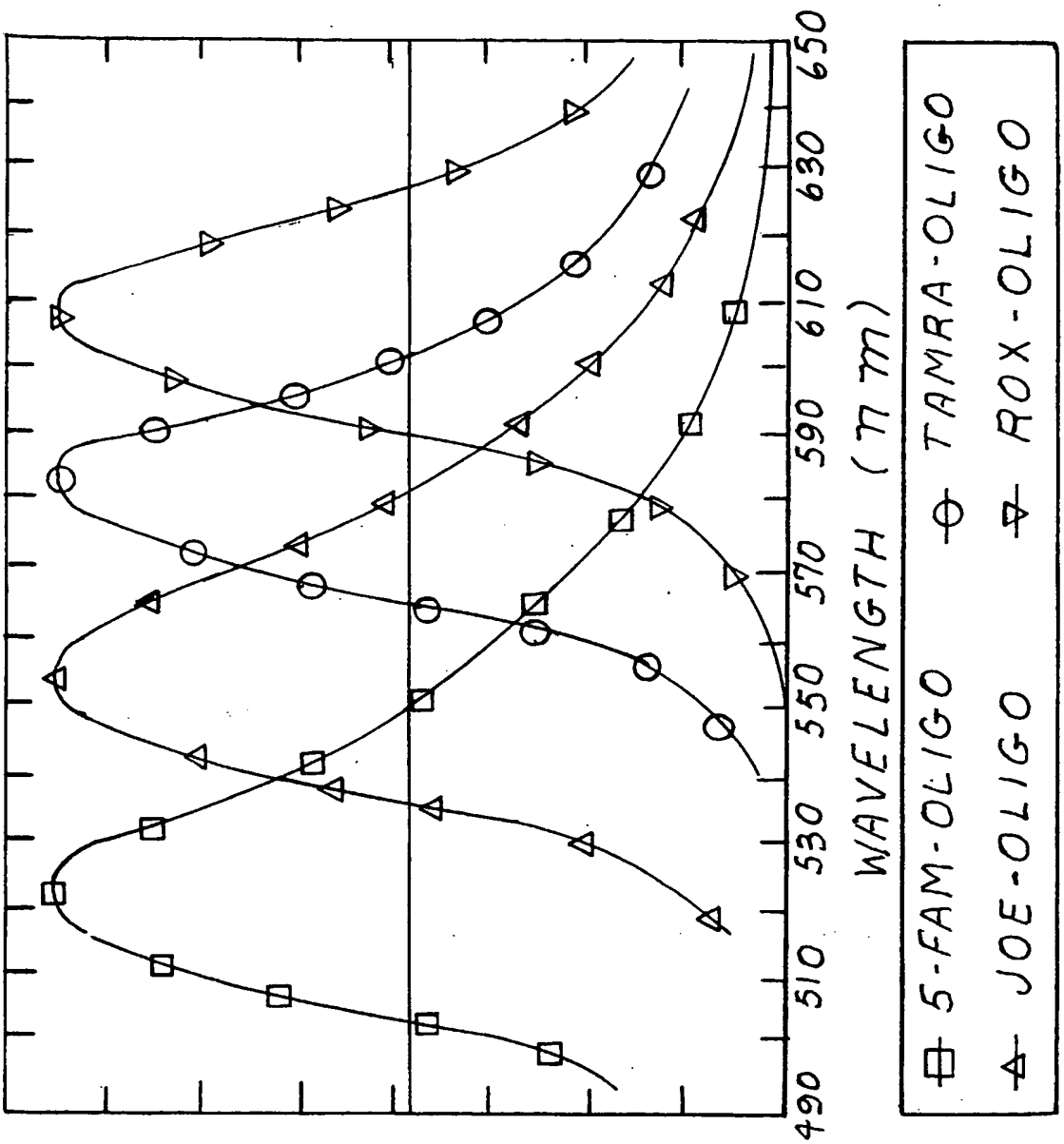


Fig. 6j.

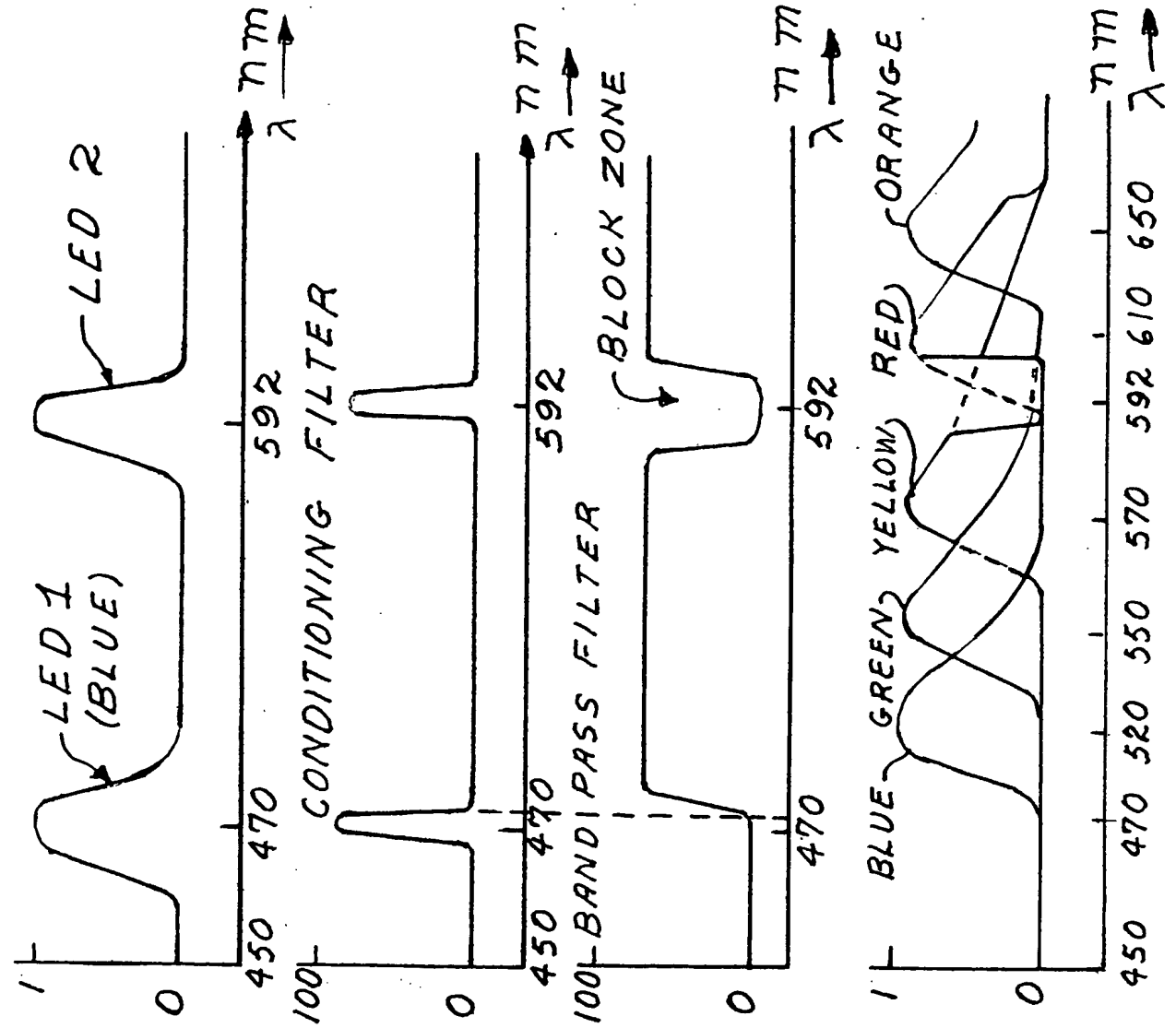


Fig. 9a. RELATIVE EXCITATION INTENSITY

Fig. 9b. % TRANSMISSON

Fig. 9c. % TRANSMISSON

Fig. 9d. RELATIVE EMISSION INTENSITY